PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 2606090/EJH	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416).
International Application No. **PCT/AU2003/000079**	International Filing Date *(day/month/year)* 24 January 2003	Priority Date *(day/month/year)* 25 January 2002
International Patent Classification (IPC) or national classification and IPC **Int. Cl.**[7] C12N 15/29 C12N 15/11 A01H 5/00 C07H 21/04		
Applicant INTERNATIONAL FLOWER DEVELOPMENTS PTY LTD et al		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 7 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheet(s).

3. This report contains indications relating to the following items:

I	☒	Basis of the report
II	☐	Priority
III	☐	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
IV	☐	Lack of unity of invention
V	☒	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
VI	☐	Certain documents cited
VII	☐	Certain defects in the international application
VIII	☒	Certain observations on the international application

Date of submission of the demand	Date of completion of the report
2 June 2003	11 March 2004
Name and mailing address of the IPEA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaustralia.gov.au Facsimile No. (02) 6285 3929	Authorized Officer **DAVID OLDE** Telephone No. (02) 6283 2569

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/AU2003/000079

I. Basis of the report

1. With regard to the **elements** of the international application:*

[X] the international application as originally filed.

[ ] the description, pages , as originally filed,
pages , filed with the demand,
pages , received on with the letter of

[ ] the claims, pages , as originally filed,
pages , as amended (together with any statement) under Article 19,
pages , filed with the demand,
pages , received on with the letter of

[ ] the drawings, pages , as originally filed,
pages , filed with the demand,
pages , received on with the letter of

[ ] the sequence listing part of the description:
pages , as originally filed
pages , filed with the demand
pages , received on with the letter of

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language which is:

[ ] the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).

[ ] the language of publication of the international application (under Rule 48.3(b)).

[ ] the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

[ ] contained in the international application in written form.

[X] filed together with the international application in computer readable form.

[ ] furnished subsequently to this Authority in written form.

[ ] furnished subsequently to this Authority in computer readable form.

[ ] The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

[X] The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

4. [ ] The amendments have resulted in the cancellation of:

[ ] the description, pages

[ ] the claims, Nos.

[ ] the drawings, sheets/fig.

5. [ ] This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

* *Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).*

** *Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report*

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims **12, 21, 27-60, 68, 69**	**YES**
	Claims **1-11, 13-20, 22-26, 61-67**	**NO**
Inventive step (IS)	Claims **27-60, 68, 69**	**YES**
	Claims **1-26, 61-67**	**NO**
Industrial applicability (IA)	Claims **1-69**	**YES**
	Claims **-**	**NO**

2. Citations and explanations (Rule 70.7)

Invention appears to reside in the discovery of Class I SAM-OMT enzymes that act on flavonoids, specifically anthocyanins, termed flavonoid methyltransferases and their use in transgenic plants to alter the colour of various parts of the plant.

Claims 1-17 relate to isolated nucleic acids encoding a flavonoid methyltransferase (FMT).

Claims 18-26 relate to genetic constructs containing a nucleic acid encoding a FMT of claims 1-3, 9.

Claims 27-35 relate to genetically modified plants comprising a nucleic acid sequence encoding an FMT of claims 1-3, 9.

Claims 36-46 relate to modified plants, parts thereof, progeny and extracts from the genetically modified plants of claims 27, 36-39, 27-42.

Claims 47-60 relate to methods of producing transgenic plants of claims 1-3, 9.

Claim 61 relates to oligonucleotides encoding a polypeptide having FMT activity.

Claim 62 is directed to oligonucleotides having specific Seq ID numbers.

Claims 63-65 relate to isolated recombinant FMT encoded by nucleic acids of claims 1-17.

Claims 66, 67 relate to prokaryotes and eukaryotes carrying plasmids encoding the FMT of claims 1-17.

Claims 68, 69 relate to the use of nucleic acids of claims 1-17 in the production of genetically modified plants.

The following documents identified in the International Search report have been considered for the purposes of this report:

D1: GenPept Accession No: AAG52015 (15 June 2001)

D2: Swiss-Prot Accession No: P93711 (15 July 1998)

D3: GenPept Accession No: CAC40584 (31 May 2001)

D4: WO2001034817 (PIONEER HI-BRED INTERNATIONAL) 17 May 2001

D5: GenPept Accession No: AAC26191 (15 July 1998)

D6: Jonsson, L.M.V. *et al.* 1982. Phytochemistry. 21(10):2457-2459

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

The scope of claims 1, 10-35, 47-49, 51-59, 63-69 and their dependent claims are not fully supported by the description. From the specification the invention appears to reside in specific Class 1 S-adenosyl-L-methionine O-methyltransferases identified by specific sequence ID, and their use in the generation of transgenic plants having altered colouration. In contrast these claims are directed to any plant flavonoid methyltransferase nucleic acid or mutant, part, fragment, portion, equivalent, analogue, derivative, or nucleic acid that is capable of hybridising to such a sequence under conditions of low stringency. As such the claims encompass material owing nothing to the applicant's invention or its teaching. Therefore as the claims encompass matter outside the scope of the applicant's invention, the claims are not fully supported by the description.

The scope of claims 39-41, 45, 46, 60 are not fully supported by the description. These claims are directed to altered plants or parts thereof, or extracts from such plants. In contrast the invention resides in specific enzymes denoted by sequence ID and their use in transgenic plants to alter plant colouration. It is not clear from these claims whether the claimed invention results from the effect of the applicant's transgene or is a result of some other interaction unconnected to the applicant's transgene. Thus as the claims are not limited to effects directly resulting from the introduction of the applicant's transgene, the scope of the claims extends beyond that of the description. Hence the claims are not fully supported.

The scope of claim 61 is not fully supported by the description. Claim 61 is directed to nucleic acids of at least 5 nucleotides having at least 50% homology or hybridising under low stringency to applicant's sequence wherein the sequence encodes a polypeptide having FMT activity. In contrast the applicant's invention resides in specific FMT enzymes as defined by sequence ID number. As claimed the nucleotide sequences are not limited to being of the same class or specific for applicant's enzymes. Therefore the scope of the claim extends beyond that of the specification. Hence claim 61 is not fully supported by the description.

The scope of claim 62 is not fully supported by the description. Claim 62 appears to be directed to small oligonucleotides identified by sequence ID. However as claimed, these nucleic acids do not appear to relate to the applicant's invention. It may be that these sequences relate to primers or probes capable of hybridising to applicant's sequences, however as claimed, they are not specific for, or to, applicant's nucleic acids encoding SAM-OMT enzymes or their use. Hence the oligonucleotides encompass material owing nothing to the applicant's invention or its teaching. Therefore as the claims encompass matter outside the scope of the applicant's invention, the claims are not fully supported by the description.

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of Box V

D7: Jonsson, L.M.V. *et al.* 1984. Planta. 160:174-179

D8: Jonsson, L.M.V. *et al.* 1983. Theoretical and Applied Genetics. 66:349-355

D9: Gauthier, A. *et al.* 1998. Archives of Biochemistry and Biophysics. 351(2):243-249

D10: Ibrahim, R.K. *et al.* 1998. Plant Molecular Biology. 36:1-10

D11: Joshi, C.P. *et al.* 1998. Plant Molecular Biology. 37:663-674

NOVELTY(N)

D1 discloses an S-adenosyl-L-methionine:trans-caffeoyl-Coenyme A 3-O-methyltransferase from *Arabidopsis* having 60% identity with Seq ID 2, 5; 61% identity with Seq ID 7, 12, 42; 65% identity with Seq ID 22; 59% identity with Seq ID 44.

Hence as claimed, claims 1, 10, 11, 13-20, 22-26, 61-67 lack novelty as all the essential features are disclosed.

D2 discloses a caffeoyl-CoA O-methyltransferase from *Populus* having 62% identity with Seq ID 2, 5; 61% identity with Seq ID 7, 12; 60% identity with Seq ID 44.

Hence as claimed, claims 1, 10, 11, 13, 14, 17-20, 22, 23, 26, 61-67 lack novelty as all the essential features are disclosed.

D3 and D4 each disclose a Zea mays protein having 63% identity with Seq ID 22.

Hence as claimed, claims 1, 15, 18, 24, 62-67 lack novelty as all the essential features are disclosed.

D5 discloses a *Eucalyptus* protein having 60.7% identity with Seq ID 42.

Hence as claimed, claims 1, 16, 18, 25, 61-67 lack novelty as all the essential features are disclosed.

D6 discloses the isolation and characterisation of a *Petunia* O-methyltransferase having methylation activity at the 3' and 5' positions of anthocyanin molecules. Said protein requires Mg^{2+} for activity and is thus a member of the Class I SAM-OMTs.

Hence claims 1-9, 61-63 lack novelty in light of this disclosure as the nucleic acid sequence of a gene or protein is considered an inherent property of that gene or protein. Thus as the protein appears to be known and as the sequence is an inherent property of the protein, these claims lack novelty in light of D6.

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of Box V

D7 discloses the isolation and characterisation of four S-adenosyl-L-methionine:anthocyanin-3', 5'-O-methyltransferases from *Petunia*. Said protein requires Mg^{2+} for activity and is thus a member of the Class I SAM-OMTs (p.177 "Properties of the four anthocyanin-methyltransferases").

Hence claims 1-9, 61-63 lack novelty in light of this disclosure as the nucleic acid sequence of a gene or protein is considered an inherent property of that gene or protein. Thus as the protein appears to be known and as the sequence is an inherent property of the protein, these claims lack novelty in light of D7.

D8 discloses that the four *Petunia* flavonoid-O-methyltransferase genes encode four separate proteins all having 3' and 5' methyltransferase activity.

Hence claims 1-9, 61-63 lack novelty in light of this disclosure as the nucleic acid sequence of a gene or protein is considered an inherent property of that gene or protein. Thus as the protein appears to be known and as the sequence is an inherent property of the protein, these claims lack novelty in light of D8.

D9 discloses two cDNA sequences encoding O-methyltransferases able to methylate both flavonoid (luteolin and quercetin at 3' position) and phenylpropanoid (caffeic and 5-hydroxyferulic acids at 3' and 5' positions) compounds.

Hence claims 1 and 2 lack novelty in light of this disclosure as all the essential features are disclosed.

Thus claims 3-69 are considered novel in light of D9.

INVENTIVE STEP(IS)

Claims 1, 9-11, 13-20, 22-26, 61-67 lack an inventive step in light of D1.

There is not considered to be any inventive merit in isolating homologues of known genes. Such procedures are routine in the art. Thus in the absence of surprising or unexpected properties, the claimed sequences and their incorporation into genetic constructs are not considered to involve an inventive step in light of D1.

Claims 1, 9, 10, 11, 13, 14, 17-20, 22, 23, 26, 61-67 lack an inventive step in light of D2.

There is not considered to be any inventive merit in isolating homologues of known genes. Such procedures are routine in the art. Thus in the absence of surprising or unexpected properties, the claimed sequences and their incorporation into genetic constructs are not considered to involve an inventive step in light of D2.

Claims 1, 15, 18, 24, 62-67 lack an inventive step in light of each of D3 and D4.

There is not considered to be any inventive merit isolating homologues of known genes. Such procedures are routine in the art. Thus in the absence of surprising or unexpected properties, the claimed sequences and their incorporation into genetic constructs are not considered to involve an inventive step in light of each of D3 and D4.

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of Box V

Claims 1, 9, 16, 18, 25, 61-67 lack an inventive step in light of D5.

There is not considered to be any inventive merit in isolating homologues of known genes. Such procedures are routine in the art. Thus in the absence of surprising or unexpected properties, the claimed sequences and their incorporation into genetic constructs are not considered to involve an inventive step in light of D5.

Claims 1-26, 61-63, 66, 67 lack an inventive step in light of D6.

There is not considered to be any inventive merit in determining the nucleic or amino acid sequence of a known protein of known function. Such procedures are routine in the art. Thus in the absence of surprising or unexpected properties, the claimed sequences and their incorporation into genetic constructs are not considered to involve an inventive step in light of D6.

Claims 1-26, 61-63, 66, 67 lack an inventive step in light of D7.

There is not considered to be any inventive merit in determining the nucleic or amino acid sequence of a known protein of known function. Such procedures are routine in the art. Thus in the absence of surprising or unexpected properties, the claimed sequences and their incorporation into genetic constructs are not considered to involve an inventive step in light of D7.

Claims 1-26, 61-63, 66, 67 lack an inventive step in light of D8.

There is not considered to be any inventive merit in determining the nucleic or amino acid sequence of a known protein of known function. Such procedures are routine in the art. Thus in the absence of surprising or unexpected properties, the claimed sequences and their incorporation into genetic constructs are not considered to involve an inventive step in light of D8.

Claims 1 and 2 lack an inventive step in light of D9.

There is not considered to be any inventive merit in isolating homologues of known genes. Such procedures are routine in the art. Thus in the absence of surprising or unexpected properties, the claimed sequences are not considered to involve an inventive step in light of D9.

D10 and D11 do not disclose or suggest applicant's flavonoid methyltransferase or its use. Hence claims 1-69 are considered novel and inventive in light of D10 and D11.

INDUSTRIAL APPLICABILITY(IA)

Claims 1-69 meet the requirements of the PCT in regard to industrial applicability.